were immunised with NM-2C5 and, after 24 and 48 hour, treated with cyclophosphamide, rendering the mice tolerant to NM-2C5 antigens. On days 18 and 39 the mice were immunised with M-4A4 and on day 42 the spleens were extracted for hybridoma production. Hybridomas were screened for reactivity towards the two cell lines using cell based ELISA and FACS analysis. Clones with a marked preference for M-4A4 were tested for biological effects and further characterized by immunohistochemistry and FACS analysis.
Results: We isolated 4 monoclonal antibodies that bound exclusively, or preferentially, to the M-4A4 cell line. FACS analysis on an array of various breast cancer cell lines showed a promising expression pattern as 3 of the antibodies reacted preferentially with cell lines know to be metastatic in vivo. One clone exhibited no binding to 20 breast cancers or 30 different normal tissues but stained cancer cells in medullary thyroid carcinoma and lung carcinoid by immunohistochemistry. Another clone inhibited the growth of the melanoma cell line MZ2 in vitro.
Conclusions: We successfully identified 3 monoclonal antibodies capable of binding exclusively to cell lines shown to be metastatic in vivo. Further studies will evaluate if the profile observed in cell lines correlates with the metastatic process on resected patient tumours, and whether the isolated antibodies are capable of inhibiting metastasis formation.

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Investigation of mitochondrial common deletion (mtDNA4977) in breast cancer tumors

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Background: Breast cancer is the most common cancer and the second most common cause of cancer-related death among women. The breast cancer progression involves the accumulation of various genetic mutations, which are present in both nuclear genomes (nDNA) and mitochondrial genomes (mtDNA). Every human cell contains between 100–1000 mitochondria with many copies of mtDNA and mutation rate of mtDNA is at least 10 times higher than that of nuclear DNA. Mutation analysis of mitochondrial DNA is helpful in the determination of developmental potential, early diagnosis and gene therapy for breast cancer. In our study, we used multiplex PCR to analyze breast cancer patients for most common mitochondrial deletion (mtDNA4977) in tissue samples and blood samples using immunohistochemical features as criteria.
Methods: Patient samples were drawn from three medical centers in Iran. We retrieved formalin-fixed, paraffin-embedded tissue blocks from women with breast cancer diagnosed, the age of 25−80 years for the years 2004 and 2005. Forty-seven samples were used for multiplex PCR and immunohistochemical diagnosis from 34 formalin-fixed and paraffin-embedded samples and 9 blood samples. CINAGEN Inc.'s DNA Extraction Kit was used to isolate blood and tissue DNA. A simple and rapid method was used to detect the simultaneous detection of mtDNA4977 deletion. Morphological and immunohistochemical diagnoses of breast cancer were retrieved from their hospital records.
Results: Five-mtDNA4977 deletions were detected by multiplex PCR in 9 breast cancers in blood samples. Comparison presence of mtDNA4977 deletion in blood samples with ER (negative) and tissue samples with ER (negative) have shown that frequency of mtDNA4977 deletions was higher in blood samples ($P < 0.001$) relatively tissue samples in breast cancer.
Conclusions: Cancer tissues are essentially free of mtDNA4977 deletions and the metabolic effect of it may be intolerable in cancer tissue but it may be minimal in blood (non-tumor) tissue. Our analysis shows testing of mtDNA4977 deletion in blood samples can be utilized as one of prognosis factors of breast cancer development risk in combination with ER.

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In vitro bioavailability testing of GGTI-2418 using the Caco-2 model

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Background: Protein prenylation is involved in the activation of a number of oncogene products. In particular, geranylgeranylated substrates of geranylgeranyl transferase I (GGTI) such as RhoA, RhoC, Rac1, R-Ras1 and R-Ras2 are known to promote tumorigenesis, metastases and invasion. On the contrary, inhibition of GGTI may impede the aberrant activation of Rho proteins and result in tumor growth inhibition and subsequent induction of apoptosis of human cancers with aberrant Rho function. GGTI-2418 (NSC 732082, MW 442) was found to be the most promising compound among the series of GGTI inhibitors, which inhibits GGTI potently (IC50 9.5 nM) and selectively over FTase (IC50 53 nM).
Methods: Caco-2 cells are commonly used as a model of mature intestinal cells. We therefore utilized an in vitro Caco-2 model to predict the bioavailability of the compound GGTI-2418. 8 μM GGTI-2418 was added to the apical side of six Caco-2-containing and six empty transwells. 282 nM 3H-mannitol (2 μCi) was also added to the apical side of six Caco-2-containing and six empty transwells. The plates were then placed in a 37ºC, 5% CO_2 incubator. The plates were removed from the incubator and 60 μL samples taken from the basolateral side of each transwell at 0.5, 1, 2, 4 and 8 hr in order to measure the levels of 3H-mannitol and GGTI-2418 that passed through the Caco-2 cells into the basolateral side of the transwells. The amount of GGTI-2418 that passed from the apical to the basolateral side of the transwell was measured using a HPLC method. The amount of 3H-mannitol passing from the apical to the basolateral side of the transwell was measured by liquid scintillation counting.
Results: A Caco-2 monolayer is considered intact and acceptable for transport studies if it exhibits a low mannitol permeability of $\leqslant$20 nm/s using approximately 4 μCi/mL in a 2 hr incubation (Caco-2 Product Sheet, In Vitro Technologies). Under these conditions, the permeability of the Caco-2 transwells used in this study was 1.93 ± 0.40 nm/s, indicating that the Caco-2 monolayer was intact and had formed very tight junctions. Over the 8 hr period of study, 23% of 3H-mannitol passed from the apical to basolateral side of empty transwells, compared to 17% of GGTI-2418. During the same time period, only 0.2% of 3H-mannitol passed from the apical to basolateral side of Caco-2 transwells, compared to 0.7% of GGTI-2418.
Conclusions: These results suggest the poor oral bioavailability of GGTI-2418 in vivo.

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Non small cell lung cancer xenografts as preclinical models for investigations with tyrosine kinase inhibitors

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Background: The epidermal growth factor receptor (EGFR) plays a crucial role in human cancer. It is involved in tumor development and progression, cell proliferation and regulation of apoptotic cell death. In lung cancer the EGFR is frequently overexpressed in 50−80% of the patients. With the tyrosine kinase inhibitors (TKI) Gefitinib and Erlotinib as well as with the monoclonal antibody Cetuximab drugs are available for the treatment of patients with lung cancer. The evaluation of clinical trials using Erlotinib and Gefitinib revealed that only a small group (adenocarcinomas, women, never-smokers and people with asian origin) did benefit from the treatment with TKIs. In addition, patients with mutations in the exon 18−21 of the EGFR gene showed a better response to therapy with TKIs.
Methods: Fresh tumor material of patients with non small cell lung cancer (NSCLC) was subcutaneously transplanted in immunodeficient mice shortly after removal. Protein analysis was performed via Western Blot analysis and immunohistochemistry with optimized protocols. DHPLC was used for mutation analysis.
Results: Up to now 102 tumors have been transplanted from which 23 passagable models were generated. It could be demonstrated that the murine passages coincide with the original tumor regarding histology, the expression of the surface proteins E-Cadherin, EpCAM, the cell proliferation marker Ki−67 and in gene profiling. The analysis of the EGFR gene revealed no mutations relating to a better response to TKIs. With the exception of two models all express a wild type EGFR. Three K-ras mutations were found in the xenografts and eight different mutations could be located in the p53 gene. Furthermore, the sensitivity of the xenografts was tested against five clinically used cytotoxic agents (Etoposid, Carboplatin, Gemcitabine, Paclitaxel and Navelbine) and two EGFR inhibitors (Erlotinib and Cetuximab). It could be shown that there exist strong differences in responses among the xenografts.
Conclusions: In summary, we have available a panel of well characterized NSCLC xenografts correlating with the clinical situation and being able to identify biomarkers and their regulation after therapeutic interventions both at genetic and at protein level.

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Elevated, cell-free mitochondrial RNA in plasma identifies a poor prognosis in prostate, head and neck, kidney and colorectal cancer patients

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Background: Quantification of circulating plasma DNA and RNA has been studied as a diagnostic marker for cancer and as prognostic marker in cancer patients. Increased levels of cell-free nucleic acids prior to treatment were found to be associated with a poor prognosis, and a decrease after